CHAPTER I

Introduction

1.1 Historical Background

Nowadays, cancer is a public health problems of the world population. The mortality rate of cancer is increasing in patients live in developing countries. Based on American Cancer Society, by the year 2013 the new cancer cases were expected about 1.6 million [1]. Furthermore, the World Health Organization (WHO) has estimated that in 2020, the world population will be over 11 million people died of cancers and more than 7 million people occurred in the economically developing countries [2]. As well as cancer has been the leading cause of death in Thailand. In 2012, Hospital-based cancer registry reported that new cases of cancer has been 4,000 in Thailand [3]. Leukemia is the tenth most common cancer in Thai people [4]. The highest incidence of leukemia in Thailand has been found in childhood [5].

Leukemia is a group of blood diseases characterized by diversity of chromosomal and molecular changes. The disease is clinically and molecularly heterogeneous characterized by the hematopoietic progenitor cells lose the ability to differentiate normally and to respond to normal regulators of proliferation [6]. The abnormal cells cannot mature beyond an early stage in life cycles. Leukemia develops to two types of white blood cells, which are lymphocytes and myelocytes, and are classified as lymphocytic leukemia and myeloid leukemia, respectively. The four types of leukemia are acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML), and chronic myelogenous leukemia (CML). Cause of leukemia has been linked to environmental and genetic risk factors, including exposure to toxic substance, radiation, carcinogenetic substance, smoking, and have genetic abnormality. These factors leading to genetic material of a white blood cell are damaged and the cell becomes malignant and capable of uncontrolled growth. In some cases of leukemia, mutation or overexpression of oncogenes or tumor suppressor genes

have expected to be biological markers for diagnosis, monitoring in the course of the disease during treatment and giving information for prognosis. The Wilms' tumor 1 (WT1) overexpression, Break point cluster region/Abelson (Bcr/Abl) and Fms-like tyrosine kinase 3 (FLT3) are good examples of biological marker in leukemia patients.

The *Wilms' tumor 1* (*WT1*) gene was initially defined as a tumor suppressor gene in pediatric kidney malignancy [7]. It is located on chromosome 11p13 [8]. The normal expression of WT1 involves in cell growth and development in hematopoiesis, including the bone marrow and lymph nodes [9]. Moreover, WT1 plays a role in the development of erythroid, myeloid, and lymphoid cells in embryonic development and adult stages [10]. The low level of WT1 protein expression is found in normal blood cells. In contrast, the high level of WT1 expression is found in leukemic cells, the average level is approximately 1,000 to 100,000 times higher than normal blood cells [11]. A previous study demonstrated an inverse correlation between WT1 expression levels and prognosis [11], increased expression of WT1 at relapse in acute leukemia [12] and growth inhibition by WT1 antisense oligomers in leukemic cells [13]. These results suggested that WT1 plays an important role in leukemogenesis as an oncogene. Furthermore, overexpression of *WT1* gene have been used as a biological marker for diagnosis and evaluation of minimal residual disease (MDR) of leukemia [14].

The *Break point cluster region/Abelson* (*Bcr/Abl*) gene was generated from reciprocal t(9;22) translocation, named Philadelphia chromosome, in which the tyrosine kinase of c-ABL is activated that promotes the growth advantage of leukemic cells [15]. The phosphorylation of Bcr/Abl oncoprotein leads to the disruption of key cellular processes. Examples include the disruption of the Ras–mitogen-activated protein kinase (MAPK) leading to increased proliferation and Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) pathway leading to impaired transcriptional activity [16]. Furthermore, chronic myeloid leukemia (CML) is characterized by finding of *Bcr/Abl* fusion gene [17]. The Philadelphia chromosome and the Bcr/Abl fusion protein are important biological markers in diagnosing and monitoring cytogenetic response to treatment in CML patients.

The *FLT3* gene encodes a class III receptor tyrosine kinase, expresses on the surface of normal hematopoietic stem/progenitor cells and most acute leukemia cells

[18]. It consists of five immunoglobulin-like domains in the extracellular region, a juxtamembrane domain, a tyrosine kinase domain, and the intracellular domain. [19]. In addition, high levels of FLT3 expression have been detected in AML and ALL blast cells [20]. Gene expression studies of leukemic cell lines have identified FLT3 ligand stimulation associated with a number of proteins and pathways that link to leukemogenesis and reduces apoptosis including the PI3 Kinase/AKT, RAS/MAPK, and STAT5 pathways [21]. Recent study showed that overexpression of the FLT3 transcript tented to be a worse prognostic factor as well as FLT3 overexpression related to the lower complete remission rate in induction chemotherapy [22]. Therefore, determination of FLT3 expression level could be served as an attractive molecular target for the treatment of leukemia.

Chemotherapy is generally effective way to treat leukemia patients. However, they also carries a negative effects because the mechanism of chemotherapeutic agents work by destroy divided cells, thus some normal cells are also destroyed by the side effect from those of agents. Nationally traditional medicine plants that have potential anticancer properties are alternative treatment for leukemia patients.

Mammea siamensis (Miq.) T. Anders. belonging to the Guttiferae family, is a Thai traditional medicine plant, namely "Saraphi". Its flowers have traditionally been used as a heart tonic, fever-lowering, and enhancement of appetite in Thailand [23]. Previous chemical studies showed that the flowers of *M. siamensis* presence several coumarins and xanthones, some of which possess potential biological and therapeutic properties [24, 25]. Recent studies showed that *M. siamensis* is a source of mammea E/BB, the active compound responsible for the inhibition of WT1 protein expression and growth rate in leukemic cells [26]. Furthermore, the isolated compounds from the flower of *M. siamensis* exert anti-proliferative effects through apoptosis induction in leukemic cells [27]**.** However, there has been no data concerning the effects of *M. siamensis* flower extracts on protein expression in leukemic cells.

1.2 Objectives

1.2.1 To investigate anti-proliferative effects of crude ethanolic extract and fractional extracts (hexane, ethyl acetate, and methanol) from *M. siamensis* flowers on Molt4, K562, and EoL-1 leukemic cell lines.

1.2.2 To determine the inhibitory effects of fractional crude ethanolic extract and fractional extracts (hexane, ethyl acetate, and methanol) from *M. siamensis* flowers on WT1, Bcr/Abl, and FLT3 expressions in Molt4, K562, and EoL-1 leukemic cell lines.

1.3 Literature Review

1.3.1 Cancer

Cancer, also called malignancy or neoplasm, is proliferative disease characterized by abnormal cells with altered growth properties. Cancer is considered to be one of the leading causes of morbidity and mortality worldwide. A major property of cancer cells is their ability to escape the anti-proliferative signals. In all types of cancer, some of the body's cells begin to divide without stopping and spreading into surrounding tissues. Most cancers start due to genetic alteration that happens over a person's lifetime. More rarely cancers start due to inherited faulty genes passed down in families. Errors in the gene can cause the cell to stop its normal function and may allow a cell to become cancerous. Possible mechanisms that can explain the uncontrolled growth of cancer cells are loss of function of tumor suppressors and/or the activation of oncogenes. In addition, gene mutations occur frequently during normal cell growth. However, cells contain a mechanism that recognizes when a mistake occurs and repairs the mistake. In the other hand, cancer cells are able to ignore signals that normally tell cells to stop dividing or that begin a process known as programmed cell death, or apoptosis. Cancer cells are also often able to evade the immune system, a network of organs, tissues, and specialized cells that protects the body from infections and other conditions. Although the immune system normally removes damaged or abnormal cells from the body, some cancer cells are able to hide from the immune system.

1.3.2 Causes of cancer

Cancer is a complex group of diseases with many possible causes. The causes of cancer were divided into two groups, environmental and genetic causes. Most cancers are related to environmental causes, including tobacco use, diet, types of infections, and environmental exposures to different types of chemicals and radiation. Besides, various cancers have been linked to the alterations of genes.

1.3.2.1 Environmental causes

Common environmental causes leading to cancer such as tobacco use, diet, types of infections, and environmental exposures to different types of chemical and radiation are risk factors for cancers. The tobacco use increases the risk of developing of cancer especially lung cancer [28]. Alcohol is believed to play a role in carcinogen that increase risk for pancreatic and oral cavity cancer [29]. Furthermore, some viruses are infectious agents that must replicate inside a host leading to promote cancer in humans. Human papilloma virus, Epstein Barr virus infection, Hepatitis B virus, and herpes virus are associated with risk for Burkitt's lymphoma, Hodgkin's lymphoma, cervical cancer, and liver cancer [30, 31]. Chemical and radiation exposures can induce some types of leukemia, skin cancer, and lymphoma and also increased risk of cancer in childhood [32].

1.3.2.2 Genetic causes

Cancer is caused by the accumulation of genetic and epigenetic mutations in genes that normally play a role in the regulation of cell proliferation. Some cases of cancer are result of mutations leading to uncontrollable cell growth. Only a small portion of cancers are the result of inherited from family members. It is possible to be born with certain genetic mutations or a fault in a gene that makes one statistically more likely to develop cancer later in life.

Numerous alterations in DNA sequence underlie the development of every neoplasm. Four key types of gene are responsible for the cell division process; oncogenes, tumor suppressor genes, genes control apoptosis, and DNA-repair genes.

1.3.2.3 Oncogene

Oncogene is a mutated version of proto-oncogene that stimulates cell proliferation cell division and cell death. The normal forms of these genes are called proto-oncogenes, while the mutated forms are called oncogenes. However, oncogenes typically exhibit increased production of proteins, leading to decreased cell differentiation, increased cell division, and inhibition of cell death [33]. Philadelphia chromosome is the best-known example of oncogenic chromosomal translocation. The fused gene is encoded a protein that exhibits high protein tyrosine kinase activity. The unregulated protein expression activates other proteins that are involved in cell cycle regulation and stimulation of cell division. As a result, the Philadelphia chromosome is associated with chronic myelogenous leukemia (CML).

1.3.2.4 Tumor suppressor genes

Tumor suppressor genes can be defined as genes which encode proteins that normally inhibit the formation of tumors. Tumor suppressor genes are normal genes that control cell division, repair DNA mistakes, and induce cell apoptosis. When tumor suppressor genes mutated, cells can grow out of control, which can contribute to cancer by inactivating that inhibitory function.

1.3.2.5 Genes control apoptosis

Programed cell death, or apoptosis, is needed to maintain homeostasis of the cell organism. Moreover, the genetic basis for apoptosis implies that cell death can be disrupted by mutation. The control of apoptosis involves many genes, e.g. the bcl-2 family and the caspase family [34]. Evidence indicated that insufficient apoptosis can manifest as cancer.

1.3.2.6 DNA-repair genes

A fourth type of gene associated with cancer is the group involved in DNA repair and maintenance of chromosome structure. Environmental factors, such as radiation, ultra violet light, and chemical agents, can damage DNA. Defection of DNA repair pathways leads to accumulate genomic alterations that cause of carcinogenesis

[35]. However, research on the correlation between DNA repair gene and cancer development have been published.

1.3.3 Leukemia

Leukemia is a malignant of blood diseases. It was first recognized by the German pathologist John Hughes Bennett in 1845 [36]. The disease is clinically and molecularly heterogeneous characterized by the hematopoietic progenitor cells lose the ability to differentiate normally and to respond to normal regulators of proliferation [6]. This result leading to decreased production and function of normal blood cells. Furthermore, leukemia cells can spread to liver, spleen, lymph nodes, and other organs.

1.3.3.1 Cause of leukemia

Causation of leukemogenesis generally involves genetic alteration and inappropriate of oncogene and tumor suppressor gene. Genetic alteration may occur spontaneously or as a result of exposure to radiation or carcinogenic substances. Leukemogenesis involve at least two major steps including development of abnormal clone due to genetic alteration or mutation and mutation of oncogene or tumor suppressor gene. There are many genes that involved in leukemogenesis. For example, *Bcr/Abl* fusion gene or Philadelphia chromosome plays a role in cell proliferation, found in CML. Other example genetic alteration in AML is internal tandem duplication of *FLT3* gene. Moreover, overexpression of another gene involved in leukemia is *WT1*.

1.3.3.2 Classification of leukemia

Leukemia is generally grouped into four categories, according to stage of cell differentiation (acute or chronic) and predominant the type of blood cell that has become cancerous (myelogenous or lymphocytic). Thus leukemia types are acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and chronic lymphocytic leukemia (CLL). Over time, leukemia cells can crowd out the normal blood cells. This can lead to serious problems such as anemia, bleeding, and infections. Leukemia cells can also spread and infiltrate to the lymph nodes or other organs.

1.3.3.2.1 Acute myelogenous leukemia (AML)

AML is a type of cancer in which the proliferation of abnormal blast cells. The myeloblasts in AML are abnormal and do not differentiate to healthy mature white blood cells. This type of cancer usually gets worse quickly if it is not treated. AML is generally a disease of older people and is uncommon before the age of 45. The average age of a patient with AML is about 67 years. AML is slightly more common among men than among women [37].

Leukemia cells can build up in the bone marrow and blood and leading to infection, anemia, or easy bleeding may occur. Furthermore, leukemia cells can metastasis outside the blood to other parts of the body, including the central nervous system (brain and spinal cord), skin, and gums. A main cause of mortality in AML patients is the defection of normal function of mature hematopoietic cells rather than the presence of numerous leukemic cells.

According to FAB classification, AML is divided to 8 major groups (Table 1.1) based on morphology to define specific immunotypes and cytochemical features. AML is categorized as undifferentiated acute myeloblastic leukemia (M0), acute myeloblastic leukemia with minimal maturation (M1), acute myeloblastic leukemia with maturation (M2), acute promyelocytic leukemia (M3), acute myelomonocytic leukemia (M4), acute monocytic leukemia (M5), acute erythroid leukemia (M6), and acute megakaryocytic leukemia (M7). Knowing the subtype of AML can be very important, as it sometimes affects both prognosis and the best treatment.

Table 1.1 The FAB classification of acute myeloblastic leukemia [40].

Acute myelocytic leukemia	**FAB classification**
M0	Myeloblastic without maturation or undifferentiated leukemia Blast > 90% of nonerythroid bone marrow cells
M1	Myeloblastic with minimal maturation Blast > 90% of nonerythroid bone marrow cells and promyelocytes < 10%
M2	Myeloblastic with maturation Myeloblastic 30-90% of nonerythroid bone marrow cells
M3	Promyelocytic Promyelocytes ≥ 30%
M4 Subtype: M4eo M4baso	Immature monocytes comprising 20-80% of of nonerythroid bone marrow cells Blast > 30%, monocyte component > 20% M4 with eosinophil > 5% M4 with basophil maturation
M5a	Monoblastic Monoblast > 80%, granulocytic component < 20%
M5b	Promonocytic Mixture of monoblast and more mature monocytic cells
M6	Erythroleukemia Erythrod precursor ≥ 50% and myeloblast > 30%
M7	Megakaryoblastic Megakaryoblast > 30%

1.3.3.2.2 Acute lymphoblastic leukemia (ALL)

ALL is a malignant disease of the bone marrow in which early lymphoid precursors proliferate and replace the normal hematopoietic cells of marrow and leukemia precursor may exhibit features of either B- or T-cell commitment. Furthermore, ALL is the most common type of childhood leukemia, affecting about 75% of kids with this leukemic cell. Kids ages 2 to 8 are more likely to be affected, but all age groups can develop ALL [38]. Because the disease progresses quickly, treatment needs to begin soon after ALL is diagnosed. The type of treatment used will depend on a number of factors including the sub-type of ALL, the genetic of the leukemic cells, and the age and general health of the person.

The FAB classification has defined three groups of ALL (L1, L2, and L3), based on morphology and heterogeneity of lymphoblast in bone marrow (Table 1.2). Sometimes the cytological features of blood film suggests only a differential diagnosis, confirmatory tests are needed. The blood film provides strong evidence of a specific diagnosis that can be indicated the appropriate direction of further tests. With advances in immunophenotyping and other techniques the role of cytochemistry in hematological diagnosis has declined considerably. These techniques are of major importance in hematological diagnosis. The main indications for immunophenotyping are to confirm a diagnosis of ALL and identify an aberrant immunophenotype that can be used to monitor minimal residual disease (MRD).

1.3.3.2.3 Chronic myelogenous leukemia (CML)

CML is a hematopoietic stem cell disease that starts in certain blood-forming cells of the bone marrow. It can be characterized by granulopoiesis, granulocytic immaturity, anemia, basophilia, thrombocytosis, and splenomegaly. The accumulation of malignant hematopoietic cells occurs in bone marrow, which may lead to bone marrow failure. CML is a fairly slow growing leukemia, but it can also change into a fast-growing acute leukemia that is hard to treat. Furthermore, CML was involved a specific chromosomal abnormality, namely Philadelphia chromosome [39]. This change forms an abnormal gene called *Bcr/Abl*, which used as biological marker for diagnosis of CML patients.

Table 1.2 The FAB classification of acute lymphoblastic leukemia [40].

FAB classification	Acute lymphoblastic leukemia		
	L1	**L2**	**L3**
Cell size	Mainly small	Large, heterogenous	Large, homoge-nous
Nuclear chromatin	Homogenous	Heterogenous	Homogenous
Nuclear shape	Regular	Irregular, clefting	Regular-oval to round
Nucleolus	Not visible	Visible	Prominent
Amount of cytoplasm	Scanty	Variable	Moderately abundant
Cytoplasmic vacuolation	Variable	Variable	prominent

Treatment options for CML patients depend on the phase of the disease, as well as factors like the age of the patient, blood counts, and if the spleen is enlarged. CML has established 3 phases of CML (chronic, accelerated, or blast phase) which are based mainly on the number of myeloblasts that are seen in the blood or bone marrow.

1.3.3.2.4 Chronic lymphocytic leukemia (CLL)

CLL is a monoclonal disorder characterized by a progressive accumulation of functionally incompetent lymphocytes in bone marrow. It is the most common form of leukemia found in adults in Western countries [41]. Some patients die rapidly, within 2-3 years of diagnosis, because of complications from CLL, but most patients live 5-10 years. Patients with CLL present with a wide range of symptoms and signs. Onset is insidious, and it is not unusual for CLL to be discovered incidentally after a blood cell count is performed for another reason; 25-50% of patients will be asymptomatic at time of presentation. Moreover, CLL patients may also develop either transformation to large cell lymphoma (Richter's syndrome) or prolymphocytic leukemia.

The CLL comprise a very heterogeneous group of neoplastic disorders. There are two staging systems used for CLL, which are known as the Rai-Sawitsky and Binet staging systems, respectively.

The Rai-Sawitsky staging system categorizes patients into low-, intermediate-, and high-risk groups, which correspond with stages 0, I, II, III, and IV, respectively.

1. Low risk (formerly stage 0): Lymphocytosis in the blood and bone marrow only (25% of presenting population)

2. Intermediate risk (formerly stages I and II): Lymphocytosis with enlarged nodes in any site or splenomegaly or hepatomegaly (50% of presentation)

3. High risk (formerly stages III and IV): Lymphocytosis with disease-related anemia (hemoglobin < 11 g/dl) or thrombocytopenia (platelets < 100×10^{9}/l) (25% of all patients)

The Binet staging system categorizes patients according to the number of lymph node groups involved (i.e., spleen and lymph nodes of neck, groin, and underarms), as well as the presence of low red blood cell count (anemia) or low number of platelets (thrombocytopenia).

1. Stage A: Hemoglobin greater than or equal to 10 g/dl, platelets greater than or equal to 100×10^{9}/l, and fewer than 3 lymph node areas involved.

2. Stage B: Hemoglobin and platelet levels as in stage A and three or more lymph node areas involved

3. Stage C: Hemoglobin less than 10 g/dl or platelets less than 100×10^{9}/l or both.

1.3.3.3 Leukemia treatment

Chemotherapy is the main form of treatment for leukemia. Initially, the aim of treatment is to destroy leukemic cells and induce a remission that there is no evidence of leukemic cells in the blood and bone marrow. Once a remission has been achieved, further treatment is needed to help destroy and any residual disease, and try to prevent relapsing in the future. However, the type and severity however will vary between individuals, depending on the type of treatment used and how the person responds to it. Furthermore, all treatments can cause side effects. The possible side effects of

chemotherapy include nausea and/or vomiting, weak, diarrhea or constipation, hair loss and thinning.

1.3.4 Genes related in leukemia

Translocations, deletions, and other nonrandom chromosomal abnormalities have been implicated in the pathogenesis of human hematologic malignant diseases [42, 43]. Most of the currently recognized genetic alterations in human leukemias result in either activation of a quiescent gene or creation of a hybrid gene encoding a chimeric protein. These genetic alterations typically relate genes that are involved in transcription and differentiation. Furthermore, many translocations that occur in leukemias affect proto-oncogenes, which are involved in cell proliferation and survival. When proto-oncogenes is constitutively expressed, resulting in overexpression of normal protein.

There are many genetic alterations that are involved in leukemogenesis. As a result of chromosome translocation, fusion gene occurs. *Bcr/Abl* fusion gene is example. This fusion gene is located on chromosome 22 or Philadelphia chromosome. It was detected in 90-95% of CML patient, which can be cytogenetic hallmark of this disease. Additionally, gene overexpression have been identified in leukemias, involving either proto-oncogene, tumor suppressor gene or signal transduction proteins. *FLT3* is good example gene that commonly affected in AML patients. These proteins are involved in signal transduction and cell proliferation. In recent study found that high levels of *FLT3* expression in hematologic malignancies have been detected in AML blasts (70%-100%) and acute lymphoblastic leukemia [20]. The other example of gene overexpression in leukemias is *WT1*. In some type of cancer, *WT1* gene was isolated as a tumor suppressor gene while an oncogenic activity was observed in leukemias.

1.3.5 Leukemic cell lines

Several human leukemia cell lines provide model systems for leukemia study including U937, HL60, K562, and Molt4 cell lines. Some characteristics of leukemic cell lines which were used in this study are provided in Table 1.3.

Table 1.3 Characteristics of leukemic cell lines [45].

Characteristics	Cell lines		
	Molt4	**K562**	**EoL-1**
Type of cell line	T-cell line	Erythroid cell line	Eosinophilic cell line
Original disease of patient	T-cell ALL	CML blast crisis	Eosinophilic leukemia
Culture medium	RPMI 1640 + 10% FBS	RPMI 1640 + 10% FBS	RPMI 1640 + 10% FBS
Properties in suspension cultures	Free-floating, single or cluster	Free-floating, single	Free-floating, single or cluster

1.3.6 Breakpoint cluster region/Abelson (Bcr/Abl)

Almost all CML patients carry a specific translocation t(9;22)(q34;q11.2) as a consequence of the Philadelphia translocation as well as encodes a 210 kDa and dysregulated tyrosine kinase which is necessary and sufficient for leukemogenesis [44]. The fusion protein affects multiple different cellular processes, including intracellular signaling, apoptosis, transcriptional regulation and cellular adhesion. Transgenic mouse models have demonstrated that *Bcr/Abl* is capable of inducing dramatic expansion of myeloid precursors *in vivo* and can produce a phenotype that resembles the human form of the disease. Several critical domains in the fusion protein have been mapped, and have provided important insights into its biological activity. The coiled-coil domain of the Bcr protein provides a dimerization motif, which promotes spontaneous Bcr/Abl dimerization leading to constitutive Abl kinase activity. Other domains implicated in oncogenicity are the SH2 domain and the C-terminal actin-binding domain, both in the Abl1 portion; these domains appear to be important for interaction with regulatory molecules and subcellular localization of the fusion protein. Numerous downstream pathways are activated by the Bcr/Abl protein, including the RAS, phosphatidylinositol 3-kinase, STAT and MAP kinase signaling cascades [39, 46]. For example, activation of the RAS pathway is thought to contribute to the increased cell division and

proliferation that have been seen in CML cells and STAT5 is implicated in the reduced apoptosis of CML progenitors [16, 47].

1.3.7 Wilms' tumor 1 (WT1)

Wilms' tumor 1 (*WT1*) gene is the gene responsible for renal pediatric malignancy or neophroblastoma, also known as Wilms' tumor. It was first described by Dr. Max Wilms in 1899. This gene is located on human chromosome 11p113 and encoded WT1 protein, which has molecular weights of about 47-58 kDa [48]. The amino acid sequence of WT1 protein revealed a presumptive transcription factor, which implicated in transcriptional repression or activation, suggesting the role of the WT1 as transcript-tion factor, with a C-terminal four Kruppel-like Cys_2-His_2 zinc fingers domain and N-terminal proline/glutamine-rich region [49]. Furthermore, the RNA splicing of WT1 mRNA results in the replacement of leucine 280 in WT1 protein by proline leading to different protein isoforms. The main four isoforms of WT1 protein have been showed to differ in their ability to bind the early growth response 1 (EGR-1) DNA consensus sequence. It may also be able to bind to different targets and have different affinities for the same DNA target [50].

It has been reported that the *WT1* gene expression has been detected in liver, fetal spleen and tissue in which hematopoiesis take place during embryonic development [51]. It is mainly found in immature cells. WT1 protein plays a role in early hemato-poiesis, apoptosis, cell differentiation and proliferation of human blood cells [52, 53].

The *WT1* gene was first defined as a tumor suppressor gene. However, it performs an oncogene rather than tumor suppressor gene function in leukemia and various types of solid tumors. *WT1* gene expression levels in leukemic cells were approximately 1,000 to 100,000 times higher than normal bone marrow and peripheral blood cells [13].

1.3.8 Fms-like tyrosine kinase 3 (FLT3)

Fms-like tyrosine kinase 3 (FLT3), also known as cluster of differentiation antigen 135 (CD135) or fetal liver kinase-2 (Flk2), is a protein receptor that in humans is encoded by the *FLT3* gene. FLT3 is a cytokine receptor which belongs to the receptor tyrosine kinase class III. It is expressed on the surface of hematopoietic progenitor cells.

FLT3 is composed of five extracellular immunoglobulin-like domains, a transmembrane domain, a juxtamembrane domain and a tyrosine-kinase domain. This initial phosphorylation event further activates the intrinsic tyrosine kinase activity, which in turn phosphorylates and activates signal transduction molecules that propagate the signal in the cell. Signal transduction through FLT3 plays a role in cell survival, proliferation, and differentiation [21, 54]. It is important for B and T cell lymphocytes development. Furthermore, signal transduction of FLT3 is important for the normal development of hematopoietic stem cells and progenitor cells. Specifically, multipotent progenitors and common lymphoid progenitors express high surface levels of FLT3. Ligand binding to FLT3 promotes receptor dimerization and subsequent signaling through phosphorylation of multiple cytoplasmic proteins, including SHC, SHP-2, SHIP, Cbl, Cbl-b, Gab1, and Gab2, as well as the activation of several downstream signaling pathways, such as the Ras/Raf/MAPK and PI3 kinase cascades [54, 55]. However, the recently studies show that the *FLT3* gene is one of the most frequently mutated genes in AML [21]. Besides, high levels of wild-type FLT3 have been reported for blast cells in 20-25% of AML patients without FLT3 mutations. These high levels may be associated with worse prognosis. Accordingly, aberrantly activated FLT3-kinase is considered to represent an attractive therapeutic target in AML.

1.3.9 *Mammea siamensis* (Saraphi)

The tree *Mammea siamensis* (Miq.) T. Anders. belongs to the Guttiferae family. It is known in Thai as "Saraphi", a small, evergreen which grows up to 15 m tall and 10-30 cm in diameter. The trunk is entirely covered with dark gray scale bark. When it is cut, it produces white gum which turns to yellow when being exposed to air. Its leaf is characterized as a simple leaf. The leaves thrive in opposite directions. The leaf is thick with ovate-oblong shape and obtuse tips. The length of leaf is 14–17 cm. and the width is 4–7 cm. The flowers are white and fragrant. It produces flowers in a single type, or in a cymose type, a type of flower that blooms in group, clustered around branches. Its flower has a 1.5 cm diameter and a lot of stamens. The flowering season was in January to March. Its fruit is of an ovate shape with acute tips, glabrous, and about 3 cm long. It is native to Myanmar, Thailand, Laos, Cambodia, and Vietnam [23]. The flowers of this

plant are used for a heart tonic, reducing of fever and enhancement of appetite in Thai traditional medicine [56].

Previous studies, several coumarins and xanthone have been isolated from the flowers of *M. siamensis* [23, 57]. In regard to cancer research, bioactive compound in many kinds of *M. siamensis* are capable of inhibiting cancer cell proliferation. The compounds from the flowers of *M siamensis* have been shown many biological activities. Recent studies revealed that isolated compounds from flower extract of *M. siamensis* showed significant antiproliferative activities against leukemia and stomach cancer cell lines [27]. Furthermore, Bioactivity-guided isolation of coumarins from *M. siamensis* flowers revealed considerable cytotoxicity of mammea A/AA, deacetyl mammea E/BA and deacetyl mammea E/BB towards human MDA-MB-231 breast cancer, U-251 brain tumor HCT-116 colon cancer, and CCRF-CEM leukemia cells [58]. The *n*-hexane fractional extract from *M. siamensis* seeds which contained a source of mammea E/BB, responsible for the inhibition of WT1 protein expression in leukemic cells [26]. However, the difference in method and solvents used in extraction resulted in the differences of the contents in extract. This also resulted in differences in biological effects of each fractional extract. Due to *M. siamensis* flowers extract wide range of biological and pharmacological effects and lack of toxicity, *M. siamensis* flowers extract were examined in this study to determine their effect on WT1, Bcr/Abl, and FLT3 expression in leukemic cell lines including Molt4, K562, and EoL-1 cells.

Figure 1.1 (A) Tree, (B) leaf, (C) flower, (D) air-dried flowers, and (E) flower powders of *M. siamensis*. (Photos by Methree Rungrojsakul, Phunsuk Anantaworasakul, and Rungkarn Sangkaruk, March 22nd, 2014)